REMARKS

Pursuant to 37 C.F.R. § 1.121, the marked-up version of the above amendments to the specification is shown in the attached Appendix.

The above amendments to the claims correct duplicative claim numbering in the application as filed.

The above amendments to the specification are made to replace incorrect nucleotide symbols pursuant to 37 CFR §§ 1.821-825. Applicants mistakenly used the symbol "E" for unknown nucleotides in SEQ. ID. Nos. 41, 42, and 50. These have been replaced by the correct symbol "N," in accord with the nucleotide symbols set forth in WIPO Standard ST.25 (1998), Appendix 2, Table 1. No new matter is introduced in this amendment.

Respectfully submitted,

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Edwin V. Merkel
Registration No. 40,087

Nixon Peabody LLP
Clinton Square, P.O. Box 31051
Rochester, New York 14603-1051
Telephone: (716) 263-1128
Facsimile: (716) 263-1600

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner of Patents and Trademarks, Washington, D.C 20231 on the date below.

6/21/01 | Jo Ann Whalen

Date | Jo Ann Whalen

APPENDIX
Marked-Up Version Showing Changes Made

The following amendments are indicated by underlined text for additions and strike-through text for deletions.

The paragraph extending from page 20, line 19 to page 21, line 4, has been replaced with the following paragraph:

The HtrVIII is a positive aerotaxis transducer in *H. salinarum* (Brooun et al., J. Bacteriol., 180:1642-1646 (1998), which is hereby incorporated by reference). A strain deleted for the *htrVIII* gene lacks positive aerotaxis while a strain overproducing the protein shows an enhanced aerotactic response. To investigate the possible role of HemAT-*Hs* and HemAT-*Bs* in aerotaxis, deletion mutants of these genes were constructed (Brooun, Ph.D thesis. University of Hawaii, Hawaii (1997), which is hereby incorporated by reference) for the construction of *hemAT-Hs* deletion strains. Construction of overexpression of *hemAT-Hs* in *H. salinarum*: *Nde*I and *Xba*I restriction sites were used to clone the *hemAT-Hs* gene into the *E. coli-H. salinarum* shuttle vector pKJ427. Top primer with *Nde*I cutting site (5'CCGAATTCCATATGAGCAACGAT AATGAC 3' (SEQ. ID. No. 40)) and bottom primer with *Xba*I cutting site (5'CCTCTA GAGGAT~~EE~~NNCTAGCTGAGCTTGCCGACC 3' (SEQ. ID. No. 41)) were synthesized and used for PCR amplification of *hemAT-Hs* gene. The PCR amplicon was cloned into TOPO cloning vector (Invitrogen) and transformed into *E. coli* competent cells. The plasmid containing *hemAT-Hs* gene in TOPO vector was subcloned into pKJ427 vector by *NdeI/Xba*I double digestion. The *hemAT-Hs*/pKJ427 construction was confirmed by PCR as well as *NdeI/Xba*I double digestion and transformed into Δ*htrVIII* strain using standard halobacteria transformation protocol. Individual colonies were checked by PCR and immunoblot to confirm the expression level of HemAT-*Hs*; Construction of OI3428: A 322 bp fragment interior to HemAT-*Bs* was amplified from the *B. subtilis* wild type strain OI1085 chromosome using primers with overhanging *Hind*III and *Bam*H1 sites (reverse primer: 5' TATGGGATCCCTTGTTCATCACGGGTCT~~E~~NTTGG 3' (SEQ. ID. No. 42), forward primer: 5' GATAAAGCTTGATCATAGCTCAGTTGACCG 3' (SEQ. ID. No. 43)). This PCR fragment was digested with *Hind*III and *Bam*H1 and cloned in the integration vector pHV501 (Vagner et al., Microbiology, 144(Pt 11):3097-3104 (1998)) to create pMK1. The resultant plasmid pMK1 was transformed into OI1085 and HemAT-*Bs* mutants were selected by erythromycin resistance. Integration of the pMKI into the correct

locus was checked by linkage analysis. The *hemAT-Bs* locus is 30% linked to the *glyk* locus as determined from the *B. subtilis* chromosomal map. GLY+ transductants were selected and scored for erythromycin resistance. Construction of OI3498: The entire HemAT-*Bs* gene including the native promoter and the ribosome binding site was amplified from the *B. subtilis* wild type strain OI1085 chromosome using primers with overhanging *EcoR*I and *BamH*I sites (HemAT-*Bs* amyup: 5' TGCTGAATTCGCAGCTTTCATTCATGTTTCCC 3' (SEQ. ID. No. 44), HemAT-*Bs* amydown: 5' TTAGGGATCCGTCAACTGATTTTTAA TTTAAGTTAC 3') (SEQ. ID. No. 45)). The PCR amplicon was digested with *EcoRI/BamH*I and cloned into the amyE integration vector pDG1730 (Guerout-Fleury et al., Gene, 180(1-2):57-61 (1996), which is hereby incorporated by reference) to produce pKZ2. The resultant plasmid pKZ2 was digested with *BglI/Xba*I to ensure a double crossover event into the *amyE* locus and then transformed into OI3428 to select for Spec-R. HemAT-*Bs* overexpression R4: Overexpression construction in *E. coli*: The HemAT-*Bs* overexpression construction was performed as follows: *B. subtilis* OI1085 genomic DNA was used for the PCR amplification of HemAT-*Bs* gene by Pfu DNA polymerase using two primers (Top primer with *BamH*I restriction site: 5'ATATGGATCCAAGGGGGATCATTGTAATGTTA TTTAAAAAAG 3' (SEQ. ID. No. 46), Bottom primer with *Pst*I site: 5' ATTACTGCAGCA ACTGATTTTTAATTTAAGTTTACATAATGAACGC 3' (SEQ. ID. No. 47)). The PCR amplicon was cloned into TOPO cloning vector (Invitrogen) and transformed into TOP 10 *E. coli* competent cells. Colonies were tested for the presence of plasmids containing the correct insert. The recombinant plasmid was digested with *BamH*I and *Pst*I and the insert with HemAT-*Bs* open reading frame was cloned into the pMALcII expression vector (New England Biolabs, Inc).

APPENDIX
Marked-Up Version Showing Changes Made

Table 3, appearing at the top of page 28 has been replaced with the following:

Table 3. Names and sequences (5' to 3') of primers used in HemAT-*Hs* truncation.

Primer Name	**Sequence (5' to 3')**	
hemAT-Hs EcoRI/NdeI top	ccgaattccatatgagcaacgataatgac	SEQ. ID. No. 48
hemAT-Hs 151 *BamHI/XbaI* bot	ctctagaggatccctagtcgtcggcaagcgcgtcc	SEQ. ID. No. 49
hemAT-Hs 250 B/X bot	cctctagaggatccentagacgtcagccatgcggtc	SEQ. ID. No. 50
hemAT-Hs 230 B/X bot	cctctagaggatccctaggcgacgtcctgcgaggtcgcc	SEQ. ID. No. 51
hemAT-Hs 210 B/X bot	cctctagaggatccctacgcgttcgccaactcctggcggc	SEQ. ID. No. 52
hemAT-Hs 190 B/X bot	cctctagaggatccctagatgtaggtgtccattgcgatc	SEQ. ID. No. 53
hemAT-Hs 170 B/X bot	cctctagaggatccctaccgggccacgagttcgtcgac	SEQ. ID. No. 54
hemAT-Hs 205 B/X bot	cctctagaggatccctactggcggctgtcgatctcgtc	SEQ. ID. No. 55
hemAT-Hs 200 B/X bot	cctctagaggatccctactcgtcgtggaggcgctgggc	SEQ. ID. No. 56
hemAT-Hs 195 B/X bot	cctctagaggatccctactgggcgtacgagtcgatgtag	SEQ. ID. No. 57
hemAT-Hs 194 B/X bot	cctctagaggatccctaggcgtacgagtcgatgtaggtgtcc	SEQ. ID. No. 58
hemAT-Hs 193 B/X bot	cctctagaggatccctagtacgagtcgatgtaggtgtcc	SEQ. ID. No. 59
hemAT-Hs 192 B/X bot	cctctagaggatccctacgagtcgatgtaggtgtccattgcg	SEQ. ID. No. 60
hemAT-Hs 191 B/X bot	cctctagaggatccctagtcgatgtaggtgtccattgcg	SEQ. ID. No. 61